REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
16-11-2015	Final Report	9-Jul-2012 - 8-Apr-2013

4. TITLE AND SUBTITLE
Final Report: Depth Resolved Nanospray Desorption Electrospray Ionization Mass Spectrometry in Biofilms

5a. CONTRACT NUMBER
W911NF-12-1-0254

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
62140-LS-II.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary system can be used to collect nanoliter sample from the biofilms at

15. SUBJECT TERMS
Depth-resolved, mass spectroscopy, biofilm, protein, microcapillary, microsensor, and microelectrode.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Haluk Beyenal
UU	UU	UU	UU		19b. TELEPHONE NUMBER: 509-335-6607

Report Title

Final Report: Depth Resolved Nanospray Desorption Electrospray Ionization Mass Spectrometry in Biofilms

ABSTRACT

The goal of this project was to develop a nano-DESI sensor which can be used to obtain in situ, depth-resolved analyses of metabolites and possibly proteins from biofilms at different depth. A microcapillary system was developed and tested for this goal. The microcapillary system delivered a solvent (ethanol) from one capillary while the other capillary collected and transported a charged liquid to the mass spectrometer inlet. We tried different configurations of microcapillary systems and optimized follow rates and its operation. The project successfully demonstrated that a dual microcapillary system can be used to collect nanoliter sample from the biofilms at different depth. However, the microcapillary system also collected cells which failed its use for MS.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Erhan Atci our graduate student who build nano-DESI sensor will include Nano-DESI in his thesis and plans to submit an abstract to present it.

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Tim Ewing	0.50	
Erhan Atci	0.40	
FTE Equivalent:	**0.90**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Haluk Beyenal	0.13	
FTE Equivalent:	**0.13**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Erhan Atci	0.40
Tim Ewing	0.50
FTE Equivalent:	**0.90**
Total Number:	**2**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See attached.

Technology Transfer

We submitted the following proposal which plans to use the technology developed in this proposal.

Development of in vitro biofilm and planktonic culture of “Ca. Liberibacter asiaticus”: a game change in HLB research. USDA, PI: David Gang of WSU. $5,000,000. ~ 1,000,000 to Beyenal group for 5 years.

We proposed to use nano-DESI (nano-capillary system) sensor to collect cells at different depth in the natural biofilms in addition to MS work.

Depth-Resolved Nanospray Desorption Electrospray Ionization Mass Spectrometry in Biofilms

Award no: W911NF1210254

FINAL PROGRESS REPORT

Statement of the problem studied

It is known that there is a wide spectrum of applications in which mass spectroscopy (MS) can provide information from a biofilm sample, and these applications can easily translate to all areas of biofilm research. The diversity of functions within the growing community of a biofilm cannot be displayed because all of the analyses are done in bulk and have no spatial resolution with respect to the biofilm. Separation techniques can be combined with MS to present a more focused data set with regard to composition; however, MS analysis still lacks the capacity to investigate variation with biofilm depth. Depth profiles will have the potential to elucidate the mechanisms of the surrounding matrix and the roles of the microorganism with respect to the biofilm layers and the growth interface. However, it has not been used for depth profiling in biofilms. This is mostly because there was no tool available to conduct these measurements in a biofilm. Our research group specializes in the development of microelectrodes and their application to biofilm systems for the depth profiling of selected chemicals. The microelectrodes we make have a several-micrometer tip diameter and can be used in a biofilm without damaging its structure. The proposed research used the techniques developed for microelectrodes to build a device that collects samples from biofilms for MS analysis. Ambient pressure surface ionization mass spectrometry is used to obtain a chemical analyte for sampling from interfaces without special sample preparation (Roach et al., 2010). Desorption electrospray ionization (DESI) is an ambient ionization technique in which charged droplets from an electrosonic spray ionization source are aimed towards a surface with a proximal atmospheric pressure mass spectrometer inlet. In this technique analyte molecules are collected from flat surfaces followed by ionization using a self-aspirating nanoelectrospray. This technique directly transports and ionizes an analyte that is desorbed from a surface into a liquid. It is called nanospray DESI (nano-DESI). The nanospray capillary transports the charged liquid to the mass spectrometer inlet directly, eliminating splashing while minimizing analyte transport distance. The goal of this project was to develop a nano-DESI sensor which can be used to obtain *in situ,* depth-resolved analyses of metabolites and possibly proteins. We proposed three tasks to achieve our goal. Task 1: Develop technology to construct dual-barrel microcapillaries and connect them to solvent and MS lines. Task 2: Optimize the solvent and MS line flow rates for biofilm applications. Task 3: Test the system for monitoring and obtaining depth-resolved mass spectra.

Summary of the most important results

Figure 1 shows developed nano-DESI sensor. This sensor addressed work related to task 1. The final configuration was slightly different than originally proposed. When we tested dual capillary system (originally proposed), we could not deliver and collect solvent at the same rates. Therefore, we tested single capillary system as shown in Figure 1. In this system the solvent and its collection was made from the same capillary. Figure 2 (left) shows a photograph of the tip of nano-DESI sensor. Figure 2 (right) shows a photograph of the nano-DESI sensor with connectors while it was operating after optimization (Task 2). We managed to operate it between 10 nL/min and 100 μL/min flow rates. After optimization of the flow rates, we tested it in biofilms. The biofilms were grown according to our previously published paper and book (Babauta and Beyenal, 2014; Lewandowski and Beyenal, 2013). However the microcapillary system was plugged by cell-clusters and stopped working (Task 3). In conclusion, we had succeeded in developing nano-DESI sensor but could not operate it with MS due to its unexpected ability to pick up cells in the biofilms. As a result, we used the nano-DESI sensor as a tool to collect cells

from the biofilms. Moreover, the microcapillary system developed for this project enabled us to develop a similar system to quantify electron transfer rates in biofilms (Babauta and Beyenal, 2014).

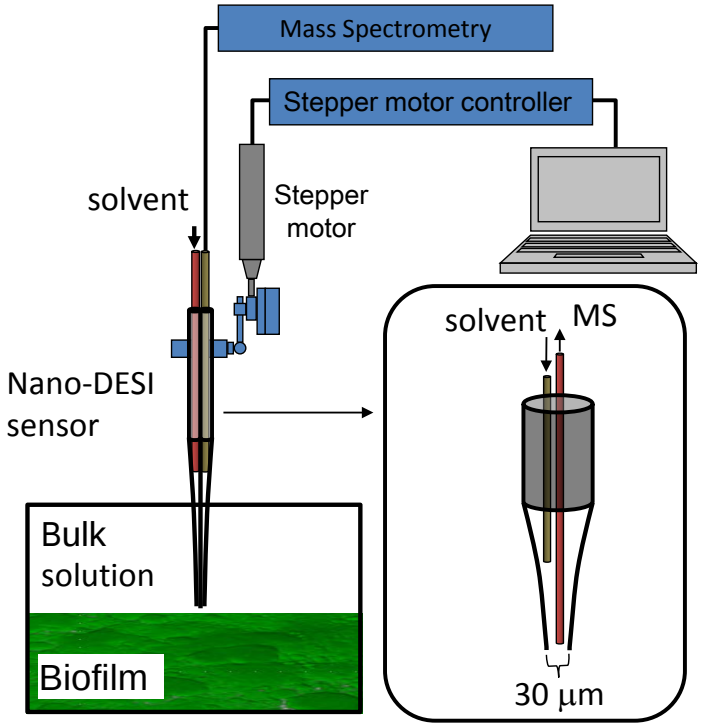
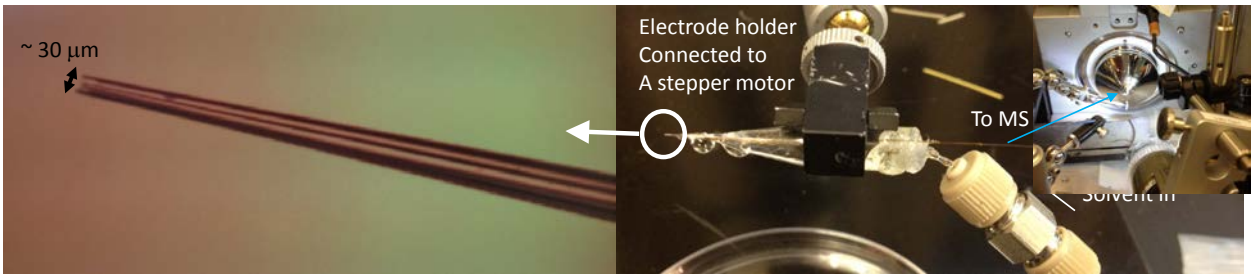


Figure 1. The configuration for nano-DESI sensor.



Tip of the capillary Operating in the lab

Figure 2. Left: A photograph of the tip of nano-DESI sensor. Right: A photograph of the nano-DESI sensor with connectors

References

Babauta, J.T., Beyenal, H., 2014. Local Current Variation by Depth in Geobacter Sulfurreducens Biofilms. Journal of the Electrochemical Society 161, H3070-H3075.

Lewandowski, Z., Beyenal, H., 2013. Fundamentals of Biofilm Research, Second Edition Edition. CRC Press, Boca Raton, FL.

Roach, P.J., Laskin, J., Laskin, A., 2010. Nanospray desorption electrospray ionization: an ambient method for liquid-extraction surface sampling in mass spectrometry. Analyst 135, 2233-2236.